

Title: Compositions and Methods for Altering Amino Acid Content of Proteins
Inventor(s): Rao *et al.*
Application No: 09/478,598
Atty Dkt N: 5718-16A (35718/193734)

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VSP HOMOLOGIES

	1	5	10	15	20	25	30
VSP-b	R	S	S	E	M	S	F
VSP-a	S	T	P	E	V	S	M
T.phos			L	K	E	T	T
Ph.vulg	D	T	E	R	A	S	M
Ar.VSP			P	N	R	S	W
Ar.1A-1			P	N	R	S	W
Ar17A-1			P	N	R	S	W
	31	35	40	45	50	55	
VSP-b	P	K	R	E	F	S	E
VSP-a	A	K	E	S	T	S	R
T.phos	C	M	K	E	P	G	K
Ph.vulg	A	A	N	E	G	A	R
Ar.VSP			E	R	S	E	R
Ar.1A-1			E	R	S	E	R
Ar17A-1			E	R	S	E	R
	60	65	70	75	80	85	
VSP-b	R	E	V	H	N	I	U
VSP-a	E	Y		P	K	T	V
T.phos	V	D	G	D	E	G	R
Ph.vulg	R	H	Y	H	E	N	V
Ar.VSP	A	A	K	N	E	V	I
Ar.1A-1	A	A	K	N	E	V	I
Ar17A-1	A	A	K	N	E	V	I
	90	95	100	105	110	115	
VSP-b	Y	E	E	N	E	E	N
VSP-a	C	V	E	K	N	S	I
T.phos	M	G	L	E	V	E	D
Ph.vulg	C	S	E	K	F	E	P
Ar.VSP	C	T	E	N	A	G	W
Ar.1A-1	C	T	E	N	A	G	W
Ar17A-1	C	T	E	N	A	G	W

TO FIG. 1A.

FIG. 1.

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FROM FIG. 1.

	120	125	130	135	140	145
VSP-b	Y	K	R	I	S	S
VSP-a	Y	N	K	E	V	S
T.phos	Y	Q	E	V	K	I
Ph.vulg	Y	N	K	E	V	S
Ar.VSP	Y	N	K	E	V	S
Ar.1A-1	Y	N	K	E	V	S
Ar17A-1	Y	N	K	E	V	S
	150	155	160	165	170	175
VSP-b	K	A	E	H	P	S
VSP-a	K	A	E	H	P	S
T.phos	N	A	S	P	D	A
Ph.vulg	K	A	E	H	P	S
Ar.VSP	K	A	E	H	P	S
Ar.1A-1	K	A	E	H	P	S
Ar17A-1	K	A	E	H	P	S
	180	185	190	195	200	205
VSP-b	R	E	N	L	R	S
VSP-a	R	E	N	L	R	S
T.phos	R	N	A	M	R	S
Ph.vulg	R	A	K	Q	R	S
Ar.VSP	R	N	S	E	R	S
Ar.1A-1	R	N	S	E	R	S
Ar17A-1	R	N	S	E	R	S
	210	218				
VSP-b	R	I	K	R	P	N
VSP-a	R	I	K	R	P	N
T.phos	R	S	E	K	R	P
Ph.vulg	R	S	E	K	R	P
Ar.VSP	R	S	E	K	R	P
Ar.1A-1	R	S	E	K	R	P
Ar17A-1	R	S	E	K	R	P

FIG. 1A.

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PROPOSED VSP β METHIONINE-ENRICHED VARIANTS

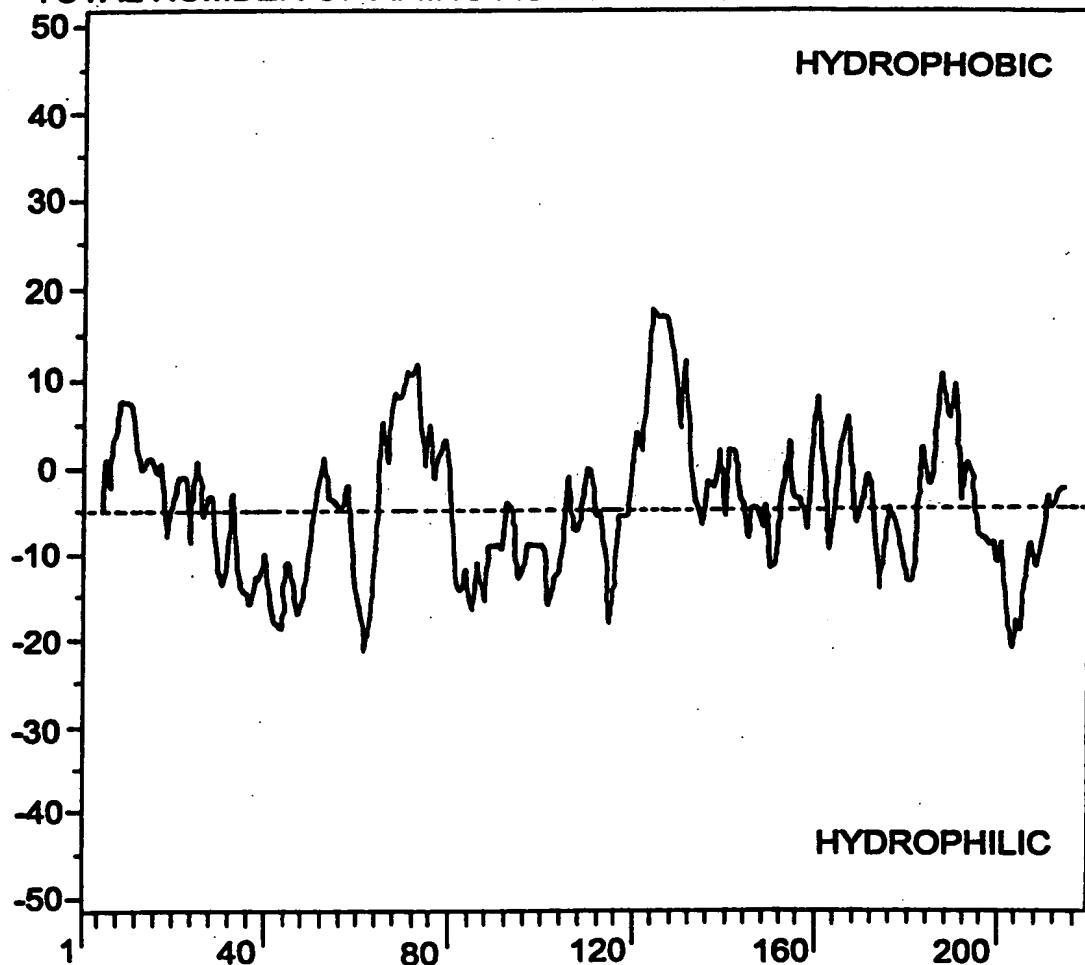
	1	5	10	15	20	25	30
VSP β	R	S	S	E	V	K	C
VSP β -Met10	M				M		M
VSP β -Met20	M				M		M
VSP β -Met30	M				M	M	M
	31	35	40	45	50	55	60
VSP β	E	P	T	K	D	Y	I
VSP β -Met10	M			M			M
VSP β -Met20	M	M	M				M
VSP β -Met30	M	M	M	M			M
	61	65	70	75	80	85	90
VSP β	E	V	H	H	N	D	I
VSP β -Met10	M	M	M	M			
VSP β -Met20	M	M	M	M			M
VSP β -Met30	M	M	M	M	M	M	M
	91	95	100	105	110	115	120
VSP β	E	E	F	I	N	T	L
VSP β -Met10				Y	D	E	W
VSP β -Met20					V	N	K
VSP β -Met30						N	K
	121	125	130	135	140	145	150
VSP β	L	S	L	G	F	K	I
VSP β -Met10	M		M			M	M
VSP β -Met20	M	M	M	M		M	M
VSP β -Met30	M	M	M	M	M	M	M
	151	155	160	165	170	175	180
VSP β	H	T	W	E	Q	L	I
VSP β -Met10							M
VSP β -Met20						M	M
VSP β -Met30					M	M	M
	181	185	190	195	200	205	210
VSP β	R	Q	G	Y	R	I	V
VSP β -Met10	M			I	I	G	D
VSP β -Met20	M			G	D	Q	W
VSP β -Met30	M				M	M	M
	211	215	218				
VSP β	P	N	P	M	Y	I	E
VSP β -Met10	M	M					
VSP β -Met20	M	M					
VSP β -Met30	M	M					

FIG. 2.

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**HYDROPATHY INDEX COMPUTATION FOR SEQUENCE VSPB.
TOTAL NUMBER OF AMINO ACIDS IS: 218.**



**HYDROPATHIC INDEX OF VSPB FROM AMINO ACID 1 TO AMINO ACID 218.
COMPUTED USING AN INTERVAL OF 9 AMINO ACIDS. (GRAVY=-4.95).**

FIG. 3A.

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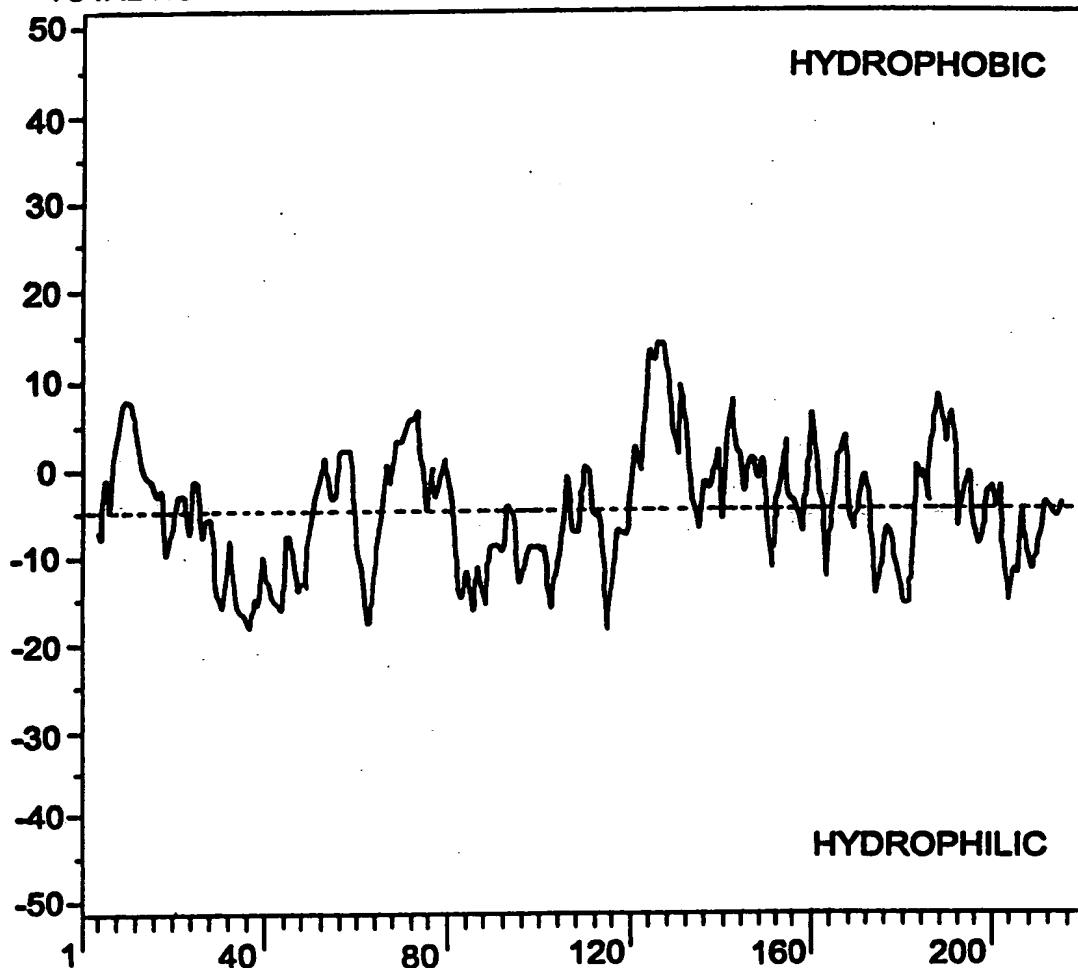
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HYDROPATHY INDEX COMPUTATION FOR SEQUENCE VSPM10.
TOTAL NUMBER OF AMINO ACIDS IS: 218



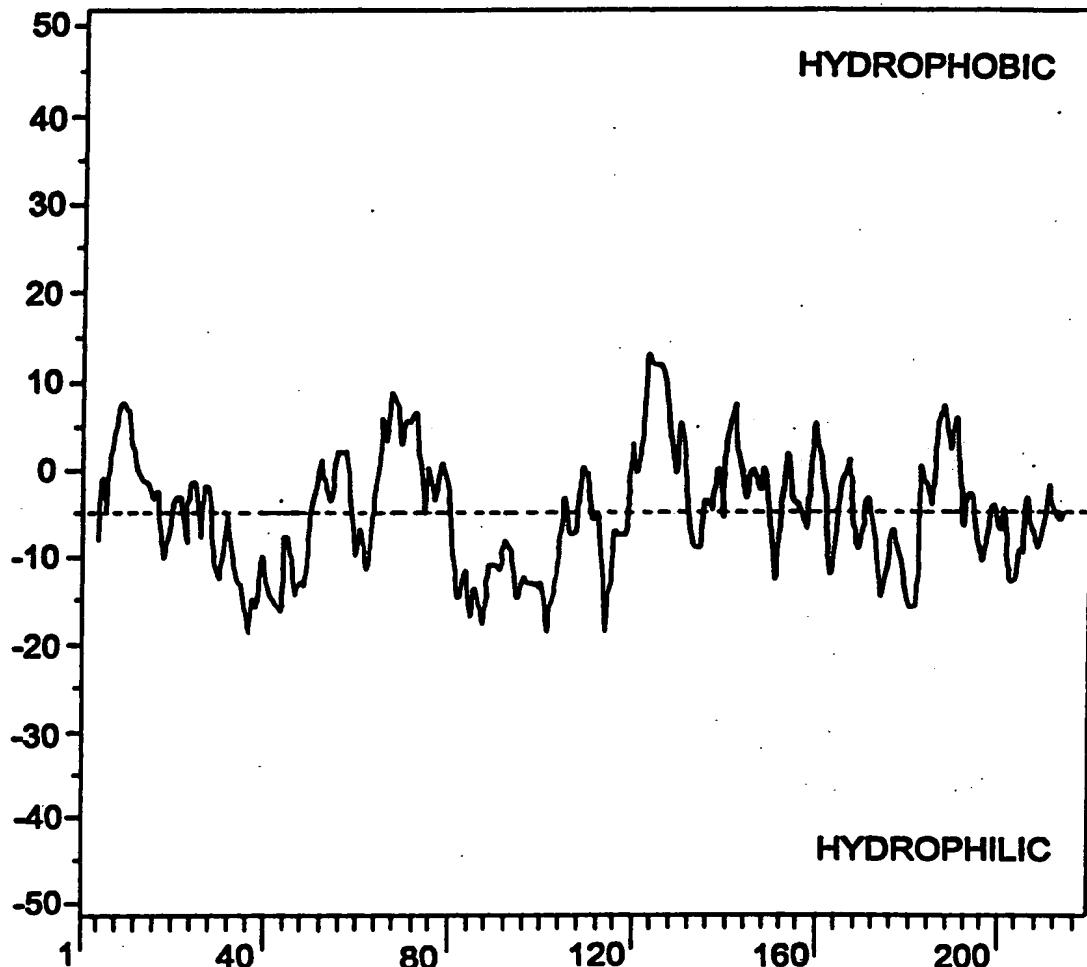
HYDROPATHIC INDEX OF VSPM1 FROM AMINO ACID 1 TO AMINO ACID 218.
COMPUTED USING AN INTERVAL OF 9 AMINO ACIDS. (GRAVY=5.52).

FIG. 3B.

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HYDROPATHY INDEX COMPUTATION FOR SEQUENCE VSPM20.
TOTAL NUMBER OF AMINO ACIDS IS: 218.



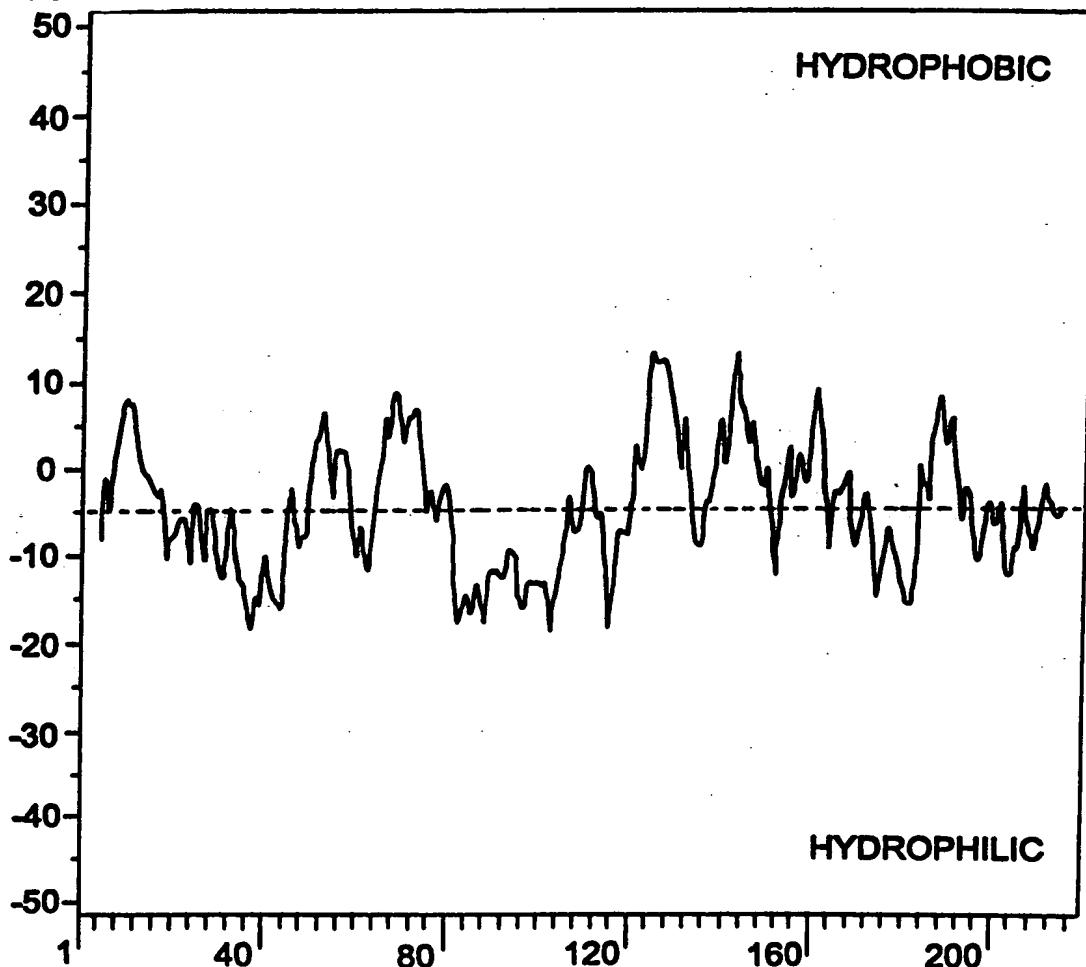
HYDROPATHIC INDEX OF VSPM20 FROM AMINO ACID 1 AMINO ACID 210.
COMPUTED USING AN INTERVAL OF 9 AMINO ACIDS. (GRAVY=-5.68).

FIG. 3C.

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HYDROPATHY INDEX COMPUTATION FOR SEQUENCE VSPM30.
TOTAL NUMBER OF AMINO ACIDS IS: 218.



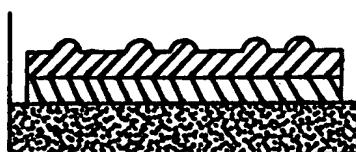
HYDROPATHIC INDEX OF VSPM30 FROM AMINO ACID 1 TO AMINO ACID 218.
COMPUTED USING AN INTERVAL OF 9 AMINO ACIDS. (GRAVY=-5.31).

FIG. 3D.

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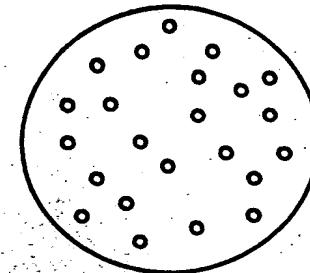
COLONY LIFT ASSAY TO DETECT PROTEIN-PROTEIN INTERACTIONS



COLONIES ON MASTER FILTER
VSPa-COATED FILTER
SB+AMP+IPTG

LAYER ANTIGEN (VSPa)-COATED FILTER AND
COLONY LIFT FILTER ON SB-IPTG-PLATE

EXPRESS VSP β MUTANTS AT 30°C

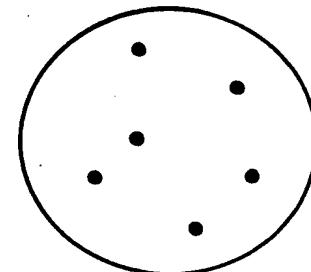


MASTER FILTER OF COLONIES
CONTAINING VSP β MUTANTS
CLONED INTO PHAGEMID VECTOR

CORRECTLY-FOLDED VSP β VARIANTS DIFFUSE THROUGH
THE MASTER FILTER AND BIND TO THE VSPa-COATED FILTER

WASH FILTER

VSPa-COATED FILTER IS INCUBATED
WITH HRP/ANTI-e TAG CONJUGATE



DEVELOPED VSPa-
COATED FILTER

DEVELOP FILTER WITH SUBSTRATE (ECL)

FIG. 5.